Plant Physiol. (1983) 73, 238–242
0032-0889/83/73/0238/05/$00.50/0

Photosynthesis and Ion Content of Leaves and Isolated Chloroplasts of Salt-Stressed Spinach[1]

Received for publication February 23, 1983 and in revised form May 28, 1983

SIMON P. ROBINSON, W. JOHN S. DOWNTON, AND JENNY A. MILLHOUSE
Commonwealth Scientific and Industrial Research Organization Division of Horticultural Research, Adelaide 5001, South Australia

ABSTRACT

Spinach (*Spinacia oleracea*) plants were subjected to salt stress by adding NaCl to the nutrient solution in increments of 25 millimolar per day to a final concentration of 200 millimolar. Plants were harvested 3 weeks after starting NaCl treatment. Fresh and dry weight of both shoots and roots was decreased more than 50% compared to control plants but the salt-stressed plants appeared healthy and were still actively growing. The salt-stressed plants had much thicker leaves. The salt-treated plants osmotically adjusted to maintain leaf turgor. Leaf K^+ was decreased but Na^+ and Cl^- were greatly increased.

The potential photosynthetic capacity of the leaves was measured at saturating CO_2 to overcome any stomatal limitation. Photosynthesis of salt-stressed plants varied only by about 10% from the controls when expressed on a leaf area or chlorophyll basis. The yield of variable chlorophyll *a* fluorescence from leaves was not affected by salt stress. Stomatal conductance decreased 70% in response to salt treatment.

Uncoupled rates of electron transport by isolated intact chloroplasts and by thylakoids were only 10 to 20% below those for control plants. CO_2-dependent O_2 evolution was decreased by 20% in chloroplasts isolated from salt-stressed plants. The concentration of K^+ in the chloroplast decreased by 50% in the salt-stressed plants, Na^+ increased by 70%, and Cl^- increased by less than 20% despite large increases in leaf Na^+ and Cl^-.

It is concluded that, for spinach, salt stress does not result in any major decrease in the photosynthetic potential of the leaf. Actual photosynthesis by the plant may be reduced by other factors such as decreased stomatal conductance and decreased leaf area. Effective compartmentation of ions within the cell may prevent the accumulation of inhibitory levels of Na^+ and Cl^- in the chloroplast.

The growth of plants is ultimately reduced by salinity although species vary in the salt concentration they can tolerate before growth is impaired (7, 10, 16). The reduction in growth is often accompanied by decreased rates of photosynthesis but the extent of this decrease depends on the concentration and type of salt, the manner in which the stress is imposed, and the relative sensitivity of the species tested (5, 7, 8, 10, 15). In a number of instances, the factors responsible for the reduction in photosynthesis have been investigated, but no clear pattern of inhibition has emerged. Many studies have concluded that the decline of photosynthesis in response to increased salinity is to some extent the result of decreased stomatal conductance. However, in several instances, decreased mesophyll conductance has also been observed suggesting inhibition of photosynthesis at the biochemical level (5, 8, 15). To identify steps in the photosynthetic pathway which are susceptible to salt stress requires investigation of component processes at the subcellular level. Photochemical reactions of isolated thylakoids (2, 24) and the activity of ribulose-1,5-bisP carboxylase (18) both have been shown to be inhibited by high concentrations of NaCl *in vitro*. However, there is little information on compartmentation of ions in higher plant cells and it is not yet clear whether such high levels of NaCl accumulate in the chloroplasts of salt-stressed plants.

In the present study, we have measured the ionic composition of leaves and intact isolated chloroplasts of salt-stressed spinach plants in relation to their photosynthetic properties. The results suggest that the compartmentation of ions within the cell prevents the accumulation of inhibitory levels of NaCl in the chloroplast and that photosynthetic potential is not significantly reduced by salinity.

MATERIALS AND METHODS

Growth of Plants. Seeds of spinach (*Spinacia oleracea* cv Hybrid 102) were germinated in moist vermiculite for 1 week at 15°C in the dark. The seedlings were transferred to a growth cabinet maintained under the following conditions: 14-h d, 25°C; night temperature, 20°C; RH, 40 to 60%; light intensity of 500 $\mu E \cdot m^{-2}\ s^{-1}$ (PAR) provided by a mixture of fluorescent and incandescent lights. After 4 d, the seedlings were transplanted into 6-L pots (2 plants/pot) of the following nutrient solution: 4 mM $Ca(NO_3)_2$, 6 mM KNO_3, 1 mM KH_2PO_4, 2 mM $MgSO_4$, 50 μM FeEDTA, 50 μM H_3BO_3, 10 μM $MnCl_2$, 0.5 μM $CuSO_4$, 0.1 μM Na_2MoO_4 and 1 μM $ZnSO_4$ made up in deionized H_2O. Three d later, the salt treatment was commenced by adding sufficient 3 M NaCl to raise the concentration in the nutrient solution by 25 mM each day, until the nutrient solution contained 200 mM NaCl. The pots were continuously aerated and were topped up daily with deionized H_2O. The plants were harvested 3 weeks after commencing salt treatment. The laminae of the second and third pair of true leaves were used for all experiments.

Experimental Analysis. Leaf water potentials were measured with thermocouple psychrometers (Wescor C52 sample chambers connected to a Wescor HR33 dewpoint microvoltmeter). Osmotic potentials were measured on the same samples following freezing and thawing and turgor pressure was calculated by difference from water potential. Stomatal conductance of the lower surface of leaves was measured with a diffusive porometer (Lambda Instruments Li60). Light intensity was measured using a quantum sensor (Lambda Instruments Li-135). Leaf thickness was measured with a micrometer or by light microscopy of hand-cut leaf sections. Chl was measured in 80% acetone according to Arnon (1). For mineral analysis, leaves were dried at 70°C and powdered in a mortar. Chloride was measured by silver ion titration with a Büchler-Cotlove chloridometer. Sodium and

[1] Partial financial support was provided by the Australian Dried Fruits Research Committee.

potassium were determined by flame emission using a Varian Techtron atomic absorption spectrophotometer. Pi was measured as described previously (20).

Induction kinetics of Chl fluorescence were measured from the upper surface of leaf discs using a fluorometer (model SF-10, Richard Branker Research) attached to a chart recorder. The leaf samples were kept in darkness for 10 min before illuminating for 4 s with red light (670 nm) at an intensity of 30 $\mu E \cdot m^{-2} s^{-1}$. This procedure was repeated twice more for each leaf disc to ensure that fluorescence yield was maximal and stable. The terminology to describe the induction curve is that adopted by Papageorgiou (19).

Leaf photosynthesis was measured in saturating CO_2 using a leaf disc O_2 electrode (4). The light intensity was 400 $\mu E \cdot m^{-2} s^{-1}$ (PAR).

Chloroplast Isolation. Intact chloroplasts were isolated by a modification of previous methods (21, 23) using solutions free of K^+, Na^+, and Cl^-. All procedures were carried out at 0°C. Leaves (50 g) were ground for 3 s in a Polytron blender with 200 ml of 330 mM sorbitol, 30 mM Mes, 2 mM ascorbate, and 0.1% BSA adjusted to pH 6.5 with Tris. The brei was squeezed through a double layer of Miracloth containing a layer of cotton wool and the filtrate was centrifuged at 1200*g* for 1 min. The pellets were resuspended in 6 ml of 330 mM sorbitol, 30 mM Hepes, and 0.2% BSA adjusted to pH 7.6 with Tris. The chloroplasts were placed in two centrifuge tubes and each was underlayered with 4 ml of resuspension medium plus 40% (v/v) Percoll. The tubes were centrifuged at 1200*g* for 1 min and then the supernatant was aspirated and the pellets resuspended in the above medium.

O_2 evolution was measured polarographically with Hansatech O_2 electrodes. The basic assay medium was 330 mM sorbitol, 2 mM EDTA, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 50 mM Hepes-KOH, pH 7.6. The percentage of intact chloroplasts was determined by measuring O_2 evolution with FeCN[2] before and after osmotic shock (14). The rate of electron transport from water to MV was determined with intact chloroplasts (15 μg Chl·ml^{-1}) plus 0.1 mM MV, 0.5 mM KCN, and 10 mM NH_4Cl. Electron transport from water to FeCN was measured with osmotically shocked chloroplasts (15 μg Chl·ml^{-1}) plus 10 mM DL-glyceraldehyde, 1 mM FeCN, and 10 mM NH_4Cl. CO_2-dependent O_2 evolution was measured with intact chloroplasts (40 μg Chl·ml^{-1}) plus 4 mM $NaHCO_3$, 0.2 mM Pi, and 1000 units·ml^{-1} catalase. Where indicated, 5 mM PPi or 2 mM PGA was also included in the assay medium.

The volume of the chloroplast stroma was determined as the sorbitol-impermeable water space (11). Intact chloroplasts were added to the assay medium with ^3H_2O (2.5 $\mu Ci \cdot ml^{-1}$) and [^{14}C] sorbitol (0.3 $\mu Ci \cdot ml^{-1}$) to give a Chl concentration of 50 $\mu g \cdot ml^{-1}$. After 30 s, 200 μl of the chloroplast suspension was added to a 0.4-ml centrifuge tube which contained 20 μl 1 N $HClO_4$ overlayered with 75 μl silicone oil (Wacker, AR180). The tubes were centrifuged and the radioactivity in the pellet and supernatant fractions was determined by liquid scintillation spectrometry.

Unless stated otherwise, all measurements reported were made at 20°C. The data in Tables I to IV represent the results of a single growth experiment. This experiment was repeated three times and in each case results similar to those reported were obtained.

RESULTS

Effect of NaCl on Growth. NaCl was introduced over a period of 8 d, in steps of 25 mM/d, to allow the plants to adjust gradually to salt treatment. The plants were then grown for a further 2 weeks in 200 mM NaCl. During the first 7 to 10 d of the experiment, growth was not visibly different to the control plants but after 14 d it was obvious that growth was retarded by the NaCl treatment. When harvested, the salt-grown plants had considerably smaller leaves and showed slight marginal chlorosis. In other respects, the plants looked healthy with no visible necrosis or leaf burn symptoms and were still actively growing. Both the fresh and dry weights of shoots were only 30% of the control plants whereas the weight of roots was 50% of the control (Table I). Because of the greater depression in shoot growth the shoot:root ratio was lower for the plants grown in NaCl on both a fresh weight and dry weight basis (Table I). The fresh weight:dry weight ratio was depressed slightly for both shoots and roots.

Table I. *Biomass of Spinach Plants Grown in Standard Nutrient Solution or with 200 mM NaCl Added*

The data are mean values ± SE for four plants.

	Control	Salt
Shoot fresh wt (g·plant^{-1})	73.1 ± 7.6	21.9 ± 1.4
Root fresh wt (g·plant^{-1})	27.6 ± 2.2	13.9 ± 1.5
Shoot dry wt (g·plant^{-1})	4.92 ± 0.40	1.56 ± 0.08
Root dry wt (g·plant^{-1})	1.03 ± 0.06	0.61 ± 0.04
Shoot:root ratio (fresh wt)	2.90 ± 0.44	1.62 ± 0.15
Shoot:root ratio (dry wt)	5.10 ± 0.73	2.61 ± 0.13
Fresh wt:dry wt ratio (shoot)	15.12 ± 0.14	14.02 ± 0.26
Fresh wt:dry wt ratio (root)	26.70 ± 0.63	23.00 ± 1.48

Table II. *Water Relations and Ionic Content of Spinach Leaves from Plants Grown with and without 200 mM NaCl*

The data are mean values ± SE. Three samples were taken for water relations, and four for other measurements, except leaf thickness which was measured for 10 leaves.

	Control	Salt
Water potential (MPa)	−0.80	−1.83 ± 0.03
Osmotic potential (MPa)	−1.15 ± 0.07	−2.35 ± 0.02
Turgor pressure (MPa)	0.35 ± 0.07	0.52 ± 0.05
Water content (% fresh wt)	93.1 ± 0.2	94.0 ± 0.3
Fresh wt (mg·cm^{-2})	56.8 ± 4.5	87.8 ± 2.9
Dry wt (mg·cm^{-2})	3.92 ± 0.34	5.25 ± 0.43
Leaf thickness (mm)	0.65 ± 0.03	1.10 ± 0.05
Leaf K^+ (mM)	264 ± 7.5	131 ± 10.9
Leaf Na^+ (mM)	9.5 ± 1.2	345 ± 10
Leaf Cl^- (mM)	2.5 ± 0.4	201 ± 23

Leaf Water Relations. The leaf water relations of control and NaCl-grown plants are presented in Table II. Addition of 200 mM NaCl to the nutrient solution decreased its osmotic potential from −0.02 to −0.83 MPa. In the NaCl-grown plants, a decrease in leaf water potential of approximately 1 MPa was associated with a 1.2 MPa decrease in osmotic potential. As a result of this, leaf turgor was somewhat higher in the salt-grown plants than in the control plants.

The most obvious morphological changes in the NaCl-grown plants were a reduction in leaf area and an increase in leaf thickness. Both fresh weight and dry weight per unit of leaf area also increased. From the levels of leaf K^+, Na^+, and Cl^- (Table II), it was estimated that most of the dry weight increase could be accounted for by the accumulation of NaCl in the leaf. Thus, the ratio of fresh weight to structural dry weight (total dry weight minus weight of $K^+ + Na^+ + Cl^-$) was increased in the salt-grown plants (data not shown). Examination of leaf sections by light microscopy suggested that an increase in cell size rather than cell number was responsible for the increased leaf thickness.

The leaves of the control plants contained more than 200 mM

[2] Abbreviations: FeCN, ferricyanide; MV, methyl viologen; PGA, 3-phosphoglycerate.

K^+ on a tissue water basis but were relatively low in Na^+ and Cl^-. Addition of 200 mM NaCl to the nutrient solution resulted in a 50% decrease in leaf K^+ and dramatic increases in Na^+ and Cl^- to levels equal to or greater than that of the nutrient solution. The accumulation of NaCl in these plants would have been sufficient to lower the osmotic potential by about 0.8 MPa. This suggests that osmotic adjustment by the leaf during salt treatment was largely a result of accumulation of electrolytes from the nutrient solution.

Leaf Photosynthetic Properties. The leaves of salt-grown plants had less Chl than the control plants (Table III). This decrease was more pronounced on a weight basis because of the increased leaf thickness but there was also less Chl per unit of leaf area. The Chl *a:b* ratio was not altered by growth in NaCl. The maximum photosynthetic capacity of leaf tissue can be measured with a leaf disc O_2 electrode (4) where any stomatal limitation of photosynthesis is removed by measurement at saturating CO_2. In this system, the photosynthetic capacity of leaves from salt-grown plants was decreased by about 10% when expressed on a leaf area basis (Table III). The rates of photosynthesis expressed on a fresh weight basis were only 55% of the control as a result of the increased leaf thickness of the salt-grown plants. Because of the lower Chl levels, the rate of photosynthesis per mg Chl was actually slightly higher for the plants grown in NaCl. However, the stomatal conductance was considerably lower than the control plants (Table III).

Some insight into the photosynthetic capabilities of leaf tissue can also be gained by measurement of Chl *a* fluorescence. The level of variable fluorescence (from I to P in the transient) and the rise time to P in leaves of salt-grown plants were not different from the control plants (Table III). There was, however, a consistent increase in the slope after P in the salt-grown plants. This slope reflects the combined effects of quenching of fluorescence by oxidation of Q and by energy-dependent quenching of fluorescence associated with the formation of ion gradients across the thylakoid membrane (13).

Isolated Chloroplasts. Intact chloroplasts were isolated from NaCl-grown and control plants and their properties are listed in Table IV. Rates of CO_2-dependent O_2 evolution by isolated chloroplasts were 50 to 80% of the values for chloroplasts from control plants, depending on the assay conditions. With Pi alone, the rate was only 50% of that for chloroplasts from control plants. However, the rate was markedly increased by PPi such that this rate was 80% that achieved by the control chloroplasts. Addition of PGA also increased the rate of O_2 evolution resulting in a rate equivalent to 80% of the control chloroplasts. These differences were consistently observed with chloroplasts isolated from plants in three separate growth experiments. The decreased rates of CO_2 fixation were not a reflection of a decreased capacity for electron transport in chloroplasts from salt-grown plants. The rate of electron transport from water to FeCN in thylakoids (prepared by osmotic shock of the intact chloroplasts) was only slightly lower than for chloroplasts from control plants and electron transport in intact chloroplasts, with MV as acceptor, was the same as control chloroplasts.

The ionic composition of the chloroplasts was also determined and from the measured chloroplast volumes it was possible to calculate the concentration of these ions in the chloroplast stroma (Table IV). Chloroplasts from control plants had K^+ levels similar to leaf levels (Table II) whereas Na^+ and Cl^- concentrations were higher in the chloroplast than in the leaf as a whole. The chloroplasts from salt-grown plants had decreased K^+ in parallel to the decrease in leaf K^+ whereas the increase in chloroplast Na^+ (70%) was much less than for leaves. The Cl^- in the chloroplasts from salt-grown plants was increased by less than 20% despite an 80-fold increase in leaf Cl^- concentration. The Pi content of the chloroplasts from salt-grown plants was decreased, being less than 50% of that in chloroplasts from control plants.

DISCUSSION

The addition of 200 mM NaCl to the nutrient solution resulted in a considerable decrease in biomass (Table I) but the plants were still healthy and actively growing. This is in agreement with the results of Coughlan and Wyn Jones (3) and suggests that under these conditions spinach is relatively tolerant of salt (*cf.* Ref. 16). The most obvious morphological difference in the salt-grown plants, apart from decreased growth, was the increase in leaf thickness (Table II). Increased succulence and leaf thickness is often observed as a result of salt treatment (3, 12, 15) and the implications for photosynthesis have been discussed by Longstreth and Nobel (15). It is obvious from the leaf water relations (Table II) that the plants had osmotically adjusted to the salt stress imposed on them; hence, any changes observed are likely to result from salinity rather than water stress. The high levels of Na^+ and Cl^- in the leaves of spinach exposed to salt stress shows that this species does not exclude NaCl, as do some salt-tolerant plants (7), but accumulates these electrolytes to provide osmotic adjustment.

Stomatal conductance was decreased by salinity (Table III) in accord with most previous studies (8, 12, 15). This suggests that leaf photosynthetic rates would have been reduced relative to the controls at normal atmospheric CO_2 concentrations. Nevertheless, the photosynthetic potential of the leaves, measured under saturating CO_2, was not greatly altered by the salt treatment when rates were expressed on a Chl or leaf area basis (Tables III and IV). The decrease in photosynthesis on a fresh weight basis has implications for the growth of the plants but is not relevant

Table III. *Photosynthetic Characteristics of Leaves from Spinach Plants Grown with or without 200 mM NaCl*

The data are mean values ± SE for four samples except for stomatal conductance which was measured for six leaves.

		Control	Salt
Chl	($mg \cdot g$ fresh wt^{-1})	1.05 ± 0.07	0.52 ± 0.02
	($\mu g \cdot cm^{-2}$)	58.7 ± 2.4	45.8 ± 0.9
	a/b ratio	3.37 ± 0.05	3.31 ± 0.06
Leaf photosynthesis	($\mu mol \cdot m^{-2}\ s^{-1}$)	27.0 ± 0.3	23.5 ± 0.4
	($\mu mol \cdot mg\ Chl^{-1}\ h^{-1}$)	167 ± 6	186 ± 6
	($\mu mol \cdot g$ fresh $wt^{-1}\ h^{-1}$)	175 ± 14	97 ± 4
Leaf fluorescence	(I to P) (relative units)	29.8 ± 1.7	31.5 ± 2.5
	Time to P (s)	2.31 ± 0.28	2.33 ± 0.36
	Slope from P (relative units·s^{-1})	4.42 ± 0.52	8.55 ± 0.58
Stomatal conductance	($cm \cdot s^{-1}$)	0.24 ± 0.02	0.07 ± 0.01

Table IV. *Properties of Chloroplasts Isolated from Spinach Plants Grown with or without 200 mM NaCl*

Rates of electron transport and CO_2-dependent O_2 evolution are expressed in $\mu mol \cdot O_2 \cdot mg\ Chl^{-1}\ h^{-1}$.

	Control	Salt
Chloroplasts intact	99%	96%
Chloroplast volume ($\mu l \cdot mg\ Chl^{-1}$)	20.7	24.2
Electron transport		
$H_2O \rightarrow FeCN$	473	433
$H_2O \rightarrow MV$	560	560
CO_2-dependent O_2 evolution		
Pi	119	60
Pi + PPi	131	104
Pi + PGA	101	82
Concentration in chloroplast (mM)		
K^+	210	121
Na^+	96	165
Cl^-	100	117
Pi	24.2	11.9

to the capability of the photosynthetic apparatus *per se*.

The measurements of variable fluorescence (Table III) support the notion that the photosynthetic apparatus of the leaves was not greatly impaired by salt treatment. The yield and rise time, both indicators of the competence of the electron transport chain, were unaffected in the salt-grown plants. Similar results have recently been reported for leaves of grapevine subjected to salt stress under conditions where osmotic adjustment had occurred (6). Changes in fluorescence have been reported following salt treatment of sunflower and bean plants (22) but it was not established whether changes in water relations, which are known to alter the fluorescence yield (6, 9) were also involved. It is not yet clear whether the increased quenching after P observed in salt-grown plants (Table III) is directly related to the photosynthetic capacity of the leaf.

In agreement with measurements on whole leaves, the photosynthetic capacity of chloroplasts isolated from the NaCl-grown plants was also not markedly inferior to those from control plants (Table IV). The electron transport rate for intact chloroplasts or thylakoids was only slightly less than the control chloroplasts, in agreement with previous results for sunflower (22). Baker (2) found that high concentrations of NaCl or KCl inhibited electron transport when added to isolated spinach thylakoids. The present results are not inconsistent with these findings since the level of Cl^- was only marginally higher in chloroplasts from salt-stressed plants and the increase in Na^+ was approximately balanced by a decrease in K^+ (Table IV). This relatively small change in total monovalent ions would not be expected to inhibit electron transport (2, 24). CO_2-dependent O_2 evolution was consistently lower in chloroplasts from salt-grown plants although the magnitude of the decrease depended on assay conditions. The highest rates, obtained when PPi plus Pi were added to the assay medium, were 80% of those for control chloroplasts which suggests that the maximum photosynthetic capacity of the chloroplasts was not drastically reduced in the salt-stressed plants.

In measuring the ion contents of isolated organelles, there is always the possibility of errors resulting from breakage of the organelles, from contamination, or from leakage of ions during isolation. We have attempted to minimize contamination and breakage of plastids by rapid purification on a Percoll gradient. The chloroplasts isolated from both control and salt-stressed plants were highly intact based on FeCN penetration and had relatively high sorbitol-impermeable spaces (Table IV), indicating retention of envelope membranes with low permeability to small molecules. Although some leakage may have occurred during isolation, the fact that ions were retained in the plastids against considerable concentration gradients suggests that the envelope membranes were also relatively impermeable to these ions. Repeated washing of isolated chloroplasts did not alter the Na^+ and Cl^- content of the chloroplasts (Robinson and Downton, unpublished) demonstrating that these ions do not readily leak out of the plastids. The photosynthetic competence of the chloroplasts supports the notion that loss of stromal contents during isolation must have been minimal.

The ionic composition of isolated chloroplasts was not the same as that in the whole leaf (Tables II and IV) suggesting compartmentation of these ions within the cell. The chloroplasts from control plants accumulated Na^+ and Cl^- to match higher concentrations than the rest of the cell whereas the concentration of K^+ was similar to that in the leaf as a whole. The level of Cl^- in chloroplasts from salt-grown plants was only slightly increased despite the large increase in leaf Cl^- suggesting that the additional Cl^- in the leaf was restricted to the vacuole. The replacement of K^+ by Na^+ in leaves was also apparent in chloroplasts but, as with Cl^-, the major part of the Na^+ increase in salt-stressed leaves was probably a result of increased vacuolar levels. In the chloroplast, the increase in Na^+ was balanced by decreased K^+ such that the total monovalent cations remained unchanged. Replacement of K^+ by Na^+ in the leaf is observed in response to salt treatment in many species (17) but the present results demonstrate that replacement also occurs in the chloroplast.

As a response to increased salinity, it has been suggested that some of the more tolerant plants accumulate Na^+ and Cl^- for osmotic adjustment but that these ions are at least partially excluded from the cytoplasm where they may inhibit metabolic function (3, 7, 10). The decreased osmotic potential in the vacuolar compartment would then be balanced by synthesis of metabolically noninhibitory organic solutes such as glycinebetaine and proline in the cytoplasm. The results of this study are consistent with such a model and indicate that the concentration of ions in the chloroplast is highly regulated, with replacement of K^+ by Na^+ taking place. Possibly as a result of this compartmentation, the photosynthetic capacity of spinach is not drastically reduced by salt stress. The response to salinity is, however, highly variable among species (7, 8, 10, 15, 16) and decreased photosynthetic capacity may occur in plants which are more sensitive to salt stress. Even in spinach, the photosynthetic capacity may not be realized in salt-stressed plants under atmospheric conditions because of stomatal limitations.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. BAKER NR 1978 Effect of high cation concentrations on photosystem II activities. Plant Physiol 62: 889–893
3. COUGHLAN SJ, RG WYN JONES 1980 Some responses of *Spinacia oleracea* to salt stress. J Exp Bot 31: 883–893
4. DELIEU T, DA WALKER 1981 Polarographic measurement of photosynthetic oxygen evolution by leaf discs. New Phytol 89: 165–178
5. DOWNTON WJS 1977 Photosynthesis in salt-stressed grape leaves. Aust J Plant Physiol 4: 183–192
6. DOWNTON WJS, J MILLHOUSE 1983 Turgor maintenance during salt stress prevents loss of variable fluorescence in grapevine leaves. Plant Sci Lett. In press
7. FLOWERS TJ, PF TROKE, AR YEO 1977 The mechanism of salt tolerance in halophytes. Annu Rev Plant Physiol 28: 89–121
8. GALE J, HC KOHL, RM HAGAN 1967 Changes in the water balance and photosynthesis of onion, bean and cotton plants under saline conditions. Physiol Plant 20: 408–420
9. GOVINDJEE, WJS DOWNTON, DC FORK, PA ARMOND 1981 Chlorophyll *a* fluorescence transient as an indicator of water potential of leaves. Plant Sci Lett 20: 191–194
10. GREENWAY H, R MUNNS 1980 Mechanisms of salt tolerance in non-halophytes. Annu Rev Plant Physiol 31: 149–190
11. HELDT HW 1980 Measurement of metabolite movement across the envelope and of the pH in the stroma and the thylakoid space in intact chloroplasts. Methods Enzymol 69: 604–613

12. HEUER B, Z PLAUT 1981 Carbon dioxide fixation and ribulose-1,5-bisphosphate carboxylase activity in intact leaves of sugar beet plants exposed to salinity and water stress. Ann Bot 48: 261-268
13. KRAUSE GH, C VERNOTTE, JM BRIANTAIS 1982 Photoinduced quenching of chlorophyll fluorescence in intact chloroplasts and algae. Resolution into two components. Biochim Biophys Acta 679: 116-124
14. LILLEY RMcC, MP FITZGERALD, KG RIENITS, DA WALKER 1975 Criteria of intactness and the photosynthetic activity of spinach chloroplast preparations. New Phytol 75: 1-10
15. LONGSTRETH DJ, PS NOBEL 1979 Salinity effects on leaf anatomy. Consequences for photosynthesis. Plant Physiol 63: 700-703
16. MAAS EV, GJ HOFFMAN 1977 Crop salt tolerance—current assessment. J Irrig Drainage Div Am Soc Civ Eng 103: 115-134
17. MARSCHNER H 1971 Why can sodium replace potassium in plants? *In* Eighth Colloquium Int. Potash Inst. Uppsala. International Potash Institute, Berne, Switzerland, pp 50-63
18. OSMOND CB, H GREENWAY 1972 Salt responses of carboxylation enzymes for species differing in salt tolerance. Plant Physiol 49: 260-263
19. PAPAGEORGIOU G 1975 Chlorophyll fluorescence: an intrinsic probe of photosynthesis. In Govindjee, ed, Bioenergetics of Photosynthesis. Academic Press, London, pp 319-371
20. ROBINSON SP 1982 3-Phosphoglycerate phosphatase activity in chloroplast preparations as a result of contamination by acid phosphatase. Plant Physiol 70: 645-648
21. ROBINSON SP 1982 Transport of glycerate across the envelope membrane of isolated spinach chloroplasts. Plant Physiol 70: 1032-1038
22. SMILLIE RM, R NOTT 1982 Salt tolerance in crop plants monitored by chlorophyll fluorescence *in vivo*. Plant Physiol 70: 1049-1054
23. WALKER DA 1980 Preparation of higher plant chloroplasts. Methods Enzymol 69: 94-104
24. WIGNARAJAH K, NR BAKER 1981 Salt induced responses of chloroplast activities in species of differing salt tolerance. Photosynthetic electron transport in *Aster tripolium* and *Pisum sativum*. Physiol Plant 51: 387-393